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Molecular Techniques Used to Identify the DNA Sequences

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ABSTRACT

Many efficient and cost-effective technologies have been developed that causes massive progress in molecular world. In this review we have discussed six methods of DNA sequencing like: 1. dideoxy sequencing; 2. cyclic array sequencing; 3. sequencing-by-hybridization; 4. nanopore sequencing; 5. Mass spectrometry and 6. Microelectrophoresis. These methods are different from each other in their efficiency cost effectiveness and several other aspects.

Introduction

In the mid -1960s, first attempt for DNA sequencing was done that was followed by Protein and RNA sequencing by analysis of degradation products (1,2). A moment came in 1977, when two groups separately led by Fred Sanger and Walter Gilbert published methods of DNA sequencing by electrophoresis with the help of single base pair resolution (3,4). The development of automated machines in 1980s for DNA sequencing was developed that results in second revolution and made the Sanger work superior to Gilbert work that was used for next few decades (5). After automation process several discoveries were made like deoxynucleotides(ddNTPs) with florescence features and thermostable polymerases enzymes results in development of DNA libraries (6,7). These discoveries lead to development of several important discoveries like Human genome project (8) and GenBank. In the recent years, several developments and changes have been made that have made the DNA sequencing easy, cost effective and accurate.

1.Dideoxy sequencing

Dideoxy sequencing is also called as Sanger sequencing in which primer initiates the process followed by polymerase that made copy of DNA strand that is complementary to the template strand whose sequence is to be determined. The copies of template strand are made either by polymerase chain reaction or by miniprep of plasmid vectors in which gene of interested has been cloned. For reaction to proceed both natural deoxynucleotides(dNTPs) and chain elongation dideoxy nucleotides (ddNTPs) have been present. In old method four separate reaction has to be carried out each of which containing one of the ddNTPs (ddATP, ddGTP, ddCTP, or ddTTP) along with polymerase enzymes, radioactive labels and dNTPs. But in recent times only one primer is used having all four ddNTPs and fluorescent dye. To lessen the number of template strands repeated cycles of denaturation, extension and annealing are performed.

Method:

The sequencing template is an unknown sequence having a known region to which a primer is attached. Sequencing (denaturation, extension and annealing) is performed by polymerase, dNTPs and ddNTPs having fluorescent dye. The product that is formed is run into capillary having denaturation polymerase that convert it into different size fragments. Then the size base separation is done by single base pair resolution with smallest fragments running fast and vice versa.

2. Cyclic array sequencing

Cyclic array is a low-cost process in which decoding is done in a two-dimensional way having millions of distinct features of sequences. The sequencing features may be randomly placed or are arranged. Each DNA feature consist of an unknown sequence along with a known universal sequence. The main feature is that all DNA sequences are not separated in individual space but they are attached and are immobilized on a single surface. A single reagent is applied and manipulate manipulated all sequences in parallel. This cyclic process interrogates all features of a base in parallel in each cycle. This cyclic process is also coupled with a fluorescent assay that acquire CDC-imaging after each cycle. After several cycles of multiplication, interrogation and imaging each feature can be analyzed easily by complete series of imaging data. This technique is low cost and can sequence million to even billions of features in a parallel in a microliter volume and its color features can account even less volume in picolitres or femtoliters.

3. Sequencing by hybridization (SBH)

Sequencing by hybridization is a method in which differential hybridization of target DNA to an array oligonucleotide is used to decode its primary DNA sequence. The success of this method depends on probe sequences that are based on the reference genome sequence that will be hybridized with target genome to reveal the difference from the reference genome. This concept has been used in many array techniques except that SBH attempts to query all bases rather than only one base on which polymorphism is defined.

Method:

For each base in target sequence there are four bases on chip that differ only in there central features/bases (dT, dA, dC ,dG) While the flanking sequence is constant and similar to reference genome. When hybridization is completed on chip, result in imaging the array, the intensity of each feature relative to others at a specific target site infer its identity.

Perlegen developed and applied SBH for resequencing non repetitive portion of 21 chromosome is some individuals results in discovery of single nucleotide polymorphisms (SNPs) (9). The main drawback of this method is that it has high percentage (3%) of false positive results, the main problem is that there is no chance of improvement of accuracy of results. In a recent study of Affymetrix array, for resequencing of *S.cerevisiae* with low false positive results from approximately 30,000 SNPs there were only eight fasle positive results (10). Nimblegen developed two tired SBH approach in which genome wise discovery of approximate location of mutation in first array and fine mapping in the second array (11,12).

4. Nanopore sequencing

This new technique of sequencing single molecule was first time used in 1980s by passing single stranded DNA from nanopore (13). This nanopore can be a biological protein (14) or a solid device (15).

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Method:

When individual nucleotide passes through the pore it obstructs the passage of electric current passing through the pore that helps in inferring the DNA sequence.

Published example of DNA sequencing using nanopore technique include: a) Measurement of loop length in DNA hair pin, duplex stem length and base-pair mismatch (16); b) classification of terminal base pair of DNA hairpin with 60-90% accuracy in single observation and 99% accuracy in 15 observations (17). Recently, a wide range of nanopore approaches are funded by NIH and are under developed.

5. Mass spectrometry (MS)

In past few decades, mass spectrometry has evolved itself into a data acquisition platform of proteomics. MS have also applications in genomics like genotyping, quantitative DNA analysis, gene expression analysis, DNA methylation and DNA/RNA sequencing (18). The method used for this purpose is matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). In this method measurement of masses DNA fragments in mixture of nucleic acid is carried out (19). In MS fragmentation is done by primer extension with dideoxy termination, and the primary goal of using MALDI-TOF-MS rather than electrophoresis for resolving DNA fragments. The fragments are transcribed to RNA and then to base specific cleavage before analysis. MS sequence is used to address the specific problems not resolved by other methods but it is unable to replace conventional methods used for DNA sequencing.

6. Microelectrophoresis

The purpose of using microelectrophoresis is the use of microfabrication techniques that were developed in semiconductor industry that causes the miniature of dideoxy sequencing (20) for example when we perform sequencing in nanoliter scales it will be converted to silicon wafers (21). A more important goal on which much of progress has been made is integration of series of sequencing steps like PCR amplification, product purification, and sequencing in a lab-on-chip form (22). Microelectrophoresis prove a very cost-effective process for sequencing and there is also key role of lab-on-chip devices for clinical molecular diagnosis.

Conclusion

Due to development of different performance parameters and precise prices of new non- sanger procedure for sequencing of DNA. There is difficulty in choosing the process according to the situation. To choose the best process following points should be keep in mind like cost per raw base, raw accuracy, cost per consensus base, consensus accuracy, read lengths, cost per read and mate-paired reads. Finally, it is said that pre-processing and post processing protocols of new techniques are not as mature as conventional dideoxy sequencing. So, we need to work on new techniques to enhance their accuracy and services.

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